bio-protocol Preprint

Not Peer Reviewed
Ask a question online

QiSant Biomarker Measurement in Urine Samples

Joshua Y.C. Yang Minnie M. Sarwal

Updated date: Jun 19, 2020

An abbreviated version of this protocol was published in Science Translational Medicine in Mar 2020

A urine score for noninvasive accurate diagnosis and prediction of kidney transplant rejection

DOI: 10.1126/scitranslmed.aba2501

Detailed protocol

Biomarker Assays

This protocol details the QiSant assay as was used for the paper "A urine score for noninvasive accurate diagnosis and prediction of kidney transplant rejection" (Yang et al., 2020) and may not reflect additional optimizations and changes since data gathering and collection. For in-house prepared reagents, quantities are given in arbitrary units (AU). All microwell plate readings were measured using a SpectraMax™ iD3 multi-mode microplate reader (Molecular Devices). All plates were washed, when necessary in the protocol, with an AquaMax™ microplate washer (Molecular Devices). In this protocol, all samples were run in duplicate.

Creatinine

Urinary creatinine was measured using the QuantiChrom™ Creatinine Assay Kit (BioAssay Systems) following manufacturer instructions.

Total Protein

Total protein was measured using the Pierce™ Coomassie Plus (Bradford) Assay Kit (Thermo Fisher Scientific) following manufacturer instructions.

Cell-free DNA (cfDNA)

1. Extract cfDNA from urine with the QIAamp Circulating Nucleic Acid Kit following manufacturer's instructions.
2. Coat the wells of a white ELISA plate with 50 µL of 1:10 diluted, extracted cfDNA or individually preprepared cfDNA standards to each well.
3. Cover the plate with adhesive foil and incubate overnight at 4°C.
4. Remove the coating solution and wash the plate three times with 400 µL PBST with full aspiration of excess liquid.
5. Block the coated wells by adding 300 µL blocking buffer (5% BSA in PBS diluted from stock reagents) per well.
6. Cover the plate with adhesive foil and incubate for 2 hours at room temperature.
7. Wash the plate three times with 400 µL PBST with full aspiration of excess liquid.
8. Add 50 µL of 10 AU/mL of the cfDNA probe. Incubate for 2 hours at room temperature.
9. Wash the plate four times with 400 µL PBST with full aspiration of excess liquid.
10. Add 50 µL of 1:200 streptavidin-HRP in 5% BSA in PBS to each well. Cover with adhesive foil and incubate for 2 hours at room temperature.
11. Wash the plate four times with 400 µL PBST with full aspiration of excess liquid.
12. Add 150 µL of SuperSignal ELISA Femto Substrate to each well. Incubate for 5 minutes at room temperature on a rotating shaker with an adhesive foil.
13. Determine the luminescent output of each well using all wavelengths.

Methylated cell-free DNA (m-cfDNA)

1. Extract cfDNA from urine with the QIAamp Circulating Nucleic Acid Kit following manufacturer's instructions.
2. Coat the wells of a white ELISA plate with 50 µL of 1:10 diluted, extracted cfDNA or individually preprepared m-cfDNA standards to each well.
3. Cover the plate with adhesive foil and incubate overnight at 4°C.
4. Remove the coating solution and wash the plate three times with 400 µL PBST with full aspiration of excess liquid.
5. Block the coated wells by adding 300 µL blocking buffer (5% BSA in PBS diluted from stock reagents) per well.
6. Cover the plate with adhesive foil and incubate for 2 hours at room temperature.
7. Wash the plate three times with 400 µL PBST with full aspiration of excess liquid.
8. Add 50 µL of 10 AU/mL of the m-cfDNA detection antibody. Incubate for 2 hours at room temperature.
9. Wash the plate four times with 400 µL PBST with full aspiration of excess liquid.
10. Add 50 µL of 1:200 streptavidin-HRP in 5% BSA in PBS to each well. Cover with adhesive foil and incubate for 2 hours at room temperature.
11. Wash the plate four times with 400 µL PBST with full aspiration of excess liquid.
12. Add 150 µL of SuperSignal ELISA Femto Substrate to each well. Incubate for 5 minutes at room temperature on a rotating shaker with an adhesive foil.
13. Determine the luminescent output of each well using all wavelengths.

CXCL10

1. Coat the wells of a clear ELISA plate with the CXCL10 capture antibody at 10 AU/mL concentration in carbonate/bicarbonate buffer (pH 9.6).
2. Cover the plate with adhesive foil and incubate overnight at 4°C.
3. Remove the coating solution and wash the plate three times with 400 µL PBST with full aspiration of excess liquid.
4. Block the coated wells by adding 300 µL blocking buffer (5% BSA in PBS diluted from stock reagents) per well.
5. Cover the plate with adhesive foil and incubate for 2 hours at room temperature.
6. Wash the plate three times with 400 µL PBST with full aspiration of excess liquid.
7. Add 250 µL of treated urine sample or individually preprepared CXCL10 standards to each well. Incubate for 2 hours at room temperature.

8. Wash the plate four times with 400 µL PBST with full aspiration of excess liquid.
9. Add 200 µL of 10 AU/mL concentration CXCL10 detection antibody to each well. Cover with adhesive foil and incubate for 2 hours at room temperature.
10. Wash the plate four times with 400 µL PBST with full aspiration of excess liquid.
11. Add 200 µL of 1-Step™ Ultra TMB-ELISA to each well. Incubate for 20 minutes at room temperature on a rotating shaker with an adhesive foil, ensuring blue color development proceeds.
12. Add 50 µL of 0.16M sulfuric acid to each well. Allow to incubate on rotating shaker for 5 minutes.
13. Determine the OD of each well at 450 nm with wavelength correction at 540 nm.

Clusterin

1. Coat the wells of a clear ELISA plate with the Clusterin capture antibody at 10 AU/mL concentration in carbonate/bicarbonate buffer (pH 9.6).
2. Cover the plate with adhesive foil and incubate overnight at 4°C.
3. Remove the coating solution and wash the plate three times with 400 µL PBST with full aspiration of excess liquid.
4. Block the coated wells by adding 300 µL blocking buffer (5% BSA in PBS diluted from stock reagents) per well.
5. Cover the plate with adhesive foil and incubate for 2 hours at room temperature.
6. Wash the plate three times with 400 µL PBST with full aspiration of excess liquid.
7. Add 250 µL of treated urine sample or individually preprepared CXCL10 standards to each well. Incubate for 2 hours at room temperature.
8. Wash the plate four times with 400 µL PBST with full aspiration of excess liquid.
9. Add 200 µL of 10 AU/mL concentration Clusterin detection antibody to each well. Cover with adhesive foil and incubate for 2 hours at room temperature.
10. Wash the plate four times with 400 µL PBST with full aspiration of excess liquid.
11. Add 200 µL of 1-Step™ Ultra TMB-ELISA to each well. Incubate for 20 minutes at room temperature on a rotating shaker with an adhesive foil, ensuring blue color development proceeds.
12. Add 50 µL of 0.16M sulfuric acid to each well. Allow to incubate on rotating shaker for 5 minutes.
13. Determine the OD of each well at 450 nm with wavelength correction at 540 nm.

Biomarker Quantification

cfDNA, m-cfDNA, total protein, CXCL10, and Clusterin assay standard curve measurements were fit with a 4-parameter logistic curve fit and sample values were interpolated to the standard curve on a per-plate basis. Duplicates were averaged for sample unknowns and concentrations were corrected for any dilutions made to the samples. Creatinine was quantified using the methodology provided by the manufacturer.

Reagents:

- Clear, 96-well flat bottom ELISA plates
- White, 96-well flat bottom ELISA plates
- Clear, 96-well flat bottom non-binding assay plates
- QuantiChrom™ Creatinine Assay Kit (BioAssay Systems)
- Pierce™ Coomassie Plus (Bradford) Assay Kit (Thermo Fisher)
- QIAamp Circulating Nucleic Acid Kit (Qiagen)
- Carbonate/Bicarbonate buffer (pH 9.6)
- PBS 1X
- PBST 0.05%
- Blocker™ BSA (10X) in PBS (Thermo Fisher)
- Streptavidin-HRP (R&D Systems)
- SuperSignal™ ELISA Femto Substrate (Thermo Fisher)
- 0.16M sulfuric acid “Stop Solution”
- 1-Step™ Ultra TMB-ELISA (Thermo Fisher)
- The following reagents are available for use by the scientific community for noncommercial purposes under an MTA with the Regents, University of California.
    - cfDNA probe, preprepared standards
    - m-cfDNA detection antibody, preprepared standards
    - CXCL10 capture antibody, detection antibody, preprepared standards
    - Clusterin capture antibody, detection antibody, preprepared standards

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Yang, J. Y. and Sarwal, M. M.(2020). QiSant Biomarker Measurement in Urine Samples. Bio-protocol Preprint. bio-protocol.org/prep342.
2. Yang, J. Y. C., Sarwal, R. D., Sigdel, T. K., Damm, I., Rosenbaum, B., Liberto, J. M., Chan-On, C., Arreola-Guerra, J. M., Alberu, J., Vincenti, F. and Sarwal, M. M.(2020). A urine score for noninvasive accurate diagnosis and prediction of kidney transplant rejection . Science Translational Medicine 12(535). DOI: 10.1126/scitranslmed.aba2501